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Molecular Ecology

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Running title (< 45 spaces including spaces)**:** Bovine tuberculosis dynamics in Michigan

Abstract (250)

The role of wildlife in the persistence and spread of livestock diseases is difficult to quantify and control. These difficulties are exacerbated when several wildlife species are potentially involved. Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, has experienced an ecological shift in Michigan, with spillover from cattle leading to an endemically infected white-tailed deer (deer) population. It has potentially substantial implications for the health and well-being of both wildlife and livestock and incurs a significant economic cost to industry and government. Deer are known to act as a reservoir of infection, with evidence of *M. bovis* transmission to sympatric elk and cattle populations. However, the role of elk in the circulation of *M. bovis* is uncertain – they are few in number, but range further than deer, so may enable long distance spread. Combining Whole Genome Sequences (WGS) for *M. bovis* isolates from exceptionally well-observed populations of elk, deer and cattle with spatio-temporal locations, we use spatial and Bayesian phylogenetic analyses to show strong spatio-temporal admixture of *M. bovis* isolates. Clustering of bTB in elk and cattle suggests either intraspecies transmission within the two populations, or exposure to a common source. However, there is no support for significant pathogen transfer amongst elk and cattle, and our data are in accordance with existing evidence that interspecies transmission in Michigan is likely only maintained by deer. This study demonstrates the value of whole-genome population studies of *M. bovis* transmission at the wildlife-livestock interface, providing insights into bTB management in an endemic system.

Introduction

Use of genomic approaches to understand disease dynamics

In recent years, whole genome sequencing (WGS) technology has created an unprecedented opportunity to study microbial populations and expand the power of traditional epidemiology. It provides insights into pathogen evolution and population structure, sources of pathogen infection, reconstruction of transmission chains, and rates of geographical spread at multiple scales (Drummond et al. 2002; Grenfell et al. 2004; Volz et al. 2009; Pybus and Rambaut 2009; Volz, Koelle, and Bedford 2013; Gire et al. 2014; Kao et al. 2014; Gardy and Loman 2018). While many studies have applied genomic approaches to understand virus evolution, the reduction in cost of WGS technologies have made feasible dense sampling of even much larger bacterial genomes. It has shown that bacterial lineages accumulate sufficient genetic variation over epidemiologically relevant timescales to generate novel insights intro transmission patterns (Biek et al. 2015). Sequence analysis tools such as Bayesian Evolutionary Analysis by Sampling Trees (BEAST) utilize the genetic variation present in sets of samples to estimate evolutionary parameters in the context of time and space (Drummond et al. 2005, 2006; Drummond and Rambaut 2007; Lemey et al. 2009). Reconstruction of pathogen genealogies from time-structured sequence data allows for the estimation of evolutionary substitution rates (molecular clock), which can be used to measure the timing of epidemiologically important events, such as epidemic outbreaks and interspecies transmission (Firth et al. 2010); they also allow us to study infectious diseases in multi-host systems and the identification of pathogen reservoirs (Heled and Drummond 2012; De Maio et al. 2015). It has been shown that ancestral state reconstruction of pathogen genealogies through phylogenetic trees is a useful tool to address this challenge (Heled and Drummond 2012). This approach allows us to estimate the probability of tree internal node states and tree branches being associated with a specific host (and as such the most likely source of infection within the sampled population), based on

relationships among the host states at the branch tips (from the sampled isolates), and has provided, for example, evidence that free-ranging elk are currently a self-sustaining brucellosis reservoir and the source of livestock infections in the Great Yellowstone Ecosystem (Kamath et al. 2016).

Control of infectious diseases at the wildlife-livestock interface

Infectious diseases at the wildlife-livestock interface endanger the health and well-being of wildlife and livestock. They contribute to considerable economic losses to each sector, including wildlife-related sectors such as hunting and wildlife tourism, and they also represent a potential burden to the whole ecosystem (Wiethoelter et al. 2015; Hassell et al. 2017). The livestock sector is affected through increased mortality and reduced livestock productivity, as well as indirect losses associated with cost of surveillance, decreased market values, food insecurity, and impacts on farmers' livelihood (Dehove et al. 2012).The recreational manipulation of the natural environment to increase the density of wildlife beyond its normal carrying capacity, together with agricultural intensification and deforestation, have resulted in interactions between wildlife and livestock becoming more frequent (Jones et al. 2013; Are R. Berentsen et al. 2014; Lavelle et al. 2016; Skuce et al. 2012; Cowie et al. 2016), creating a dynamic and bidirectional opportunity for pathogens to circulate freely within and across species (Bengis, Kock, and Fischer 2002), via direct and/or indirect routes (use of communal environment, shared resources, etc). The control of infectious diseases at the wildlife-livestock interface is particularly challenging because of the differences in disease control efforts aimed respectively at both livestock and wildlife populations (Gortazar et al. 2015; Bird and Mazet 2018), as these are usually managed by different organisational entities (Miller, Farnsworth, and Malmberg 2013; Welburn 2011; Mcbeth and Shanahan 2004).

Bovine tuberculosis in a multi-host system in Michigan

Michigan, USA, is one of many places worldwide where the zoonotic disease bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, has become established in free-ranging wildlife (S D Fitzgerald and Kaneene 2013; Palmer 2013; Gortázar, Che Amat, and O'Brien 2015), complicating eradication and control of the disease in cattle. In areas where more than one sympatric wildlife species may be capable of acting as a competent reservoir, determining the roles of the different species in disease maintenance can be both difficult and important, reflecting problems found in many other systems (Haydon et al. 2002; Hlokwe, van Helden, and Michel 2014; Nugent, Gortazar, and Knowles 2015; Shury 2015).

In Michigan, while white-tailed deer (*Odocoileus virginianus*; deer) are well-established as the primary wildlife maintenance host of bTB (Schmitt et al. 1997; O'Brien et al. 2006, 2011; Palmer 2013). In areas where they are sympatric with infected elk (*Cervus elaphus nelsoni*), some uncertainty remains concerning what role, if any, elk play in the epidemiology of the disease (O'Brien et al. 2008). While elk have thus far been assumed to be spillover hosts due to the small number of *M. bovis*-positive animals found to date, they have proven to be capable maintenance hosts in other settings (Fanning and Edwards 1991; Rhyan et al. 1992; Shury and Bergeson 2011). If elk were maintenance hosts in Michigan, management objectives for the population would likely need to shift from conservation for sustained use (hunting and recreational viewing) to disease control. Furthermore, if elk populations are not acting as maintenance hosts they could still play an important role in disease persistence and spread, due to their wide-ranging behaviour relative to deer (Walsh 2007). Evidence for either could entail the need for measures such as density reductions, or issuance of out-of-season shooting permits for animals in close proximity to livestock operations, and exacerbate any social and political conflicts that may exist between wildlife and agricultural interests (O'Brien, Cook, Schmitt,

and Jessup 2014). Moreover, the resources necessary to provide bTB surveillance could escalate disproportionately (Livingstone et al. 2015). Ongoing surveillance of bTB in deer and elk populations has provided valuable information on the prevalence and spatial occurrence of bTB in areas of Michigan where the two species are sympatric. This provides an ideal background for using WGS to identify genetic clustering of isolates. This would be indicative of intraspecies transmission, potentially revealing evidence of maintenance of *M. bovis* in elk, and allowing for estimation of interspecies transmission rates amongst the sampled elk, deer and cattle (*Bos taurus*) populations (Kao et al. 2014).

Objectives

In this study, we evaluate the spatial and temporal dynamics of bTB amongst wildlife and livestock in the Lower Peninsula of Michigan. We use WGS to create high resolved time calibrated phylogenies and generate a robust genomic dataset with temporal, spatial and host phenotypic data. Our objectives for this study are to: i) investigate the evolutionary dynamics of *M. bovis* in the Michigan Lower Peninsula; ii) identify *M. bovis* lineages associated with host species and/or geographic locations; iii) quantify the probability *M. bovis* transmission between host species; and iv) gain insights into the needs of a new management program of bTB control at the wildlife-livestock interface. We present data showing three genetically distinct *M. bovis* clades with variable temporal, host and geographical distributions. While elk is present in two out of three clades, no evidence was found for significant transmission between cattle and elk. Our analyses are also consistent with interspecies transmission in Michigan being maintained by deer, and thus the major management focus should continue to be in controlling the disease in the endemic deer population. This study shows the value of WGS for examining bacterial pathogen transmission at the wildlife-livestock interface.

176 **Materials and Methods**

177 1. **Data.** *Mycobacterium bovis* isolates were obtained from naturally infected wildlife (deer
178 and elk) and livestock (cattle) tissue samples using standard isolation protocols (Parish and
179 Stocker 2002). Wildlife management information, surveillance methods used to find infected
180 free-ranging wildlife (through hunting and out-of-season shooting permits) and hunting
181 territories (from where the data were collected) are described in Text S2 and elsewhere
182 (O'Brien et al. 2002, 2004, 2008; MDNR1 2018; MDNR2 2018), as are the origin of cattle
183 isolates (Tsao et al. 2014). Because we are focusing on the potential role of elk in the
184 transmission of bTB amongst the three species, bTB-positive deer that were spatially (within
185 10 miles of the sampling location of an elk) and temporally close (within three years before or
186 after the sampled elk date) to each positive elk were selected for inclusion from among the
187 available archived isolates. The choice of these thresholds was based on the size of the elk's
188 home range and on the deer's average lifespan in the wild. Different research projects in
189 Michigan have looked at elk home range use (Ruhl 1984; Beyer 1987; Walsh 2007) and have
190 found that home ranges of individual elk are highly variable, ranging from 2 to 100 square
191 miles. It has been shown that there are no habitat barriers to the movement of elk that would
192 create subpopulations, and therefore there is evidence for only a single elk group (Walsh
193 2007). To enhance the likelihood of selecting isolates from deer that have been in contact with
194 elk, we chose the upper end of the elk ranges and selected all deer isolates that were within a
195 10-mile radius of each elk (encompassing a total area of ~ 314.6 square miles). The average
196 lifespan of captive deer is 14 years, but in the wild it is typically only two (Tullar 1983),
197 therefore, we chose a 3-year window around each elk isolate date to improve the opportunities
198 to capture evidence of direct contact. As we expect animals living in close spatial and
199 temporal proximity to be more likely to share the same *M. bovis* strains and, should elk and
200 deer transmit bTB freely between them, this approach would optimize the opportunities to

generate well-mixed phylogenies. Some individual elk range further than the core elk range (elk core range and hunting management units are shown in Figures 1 and S1, respectively); therefore, for the cases where isolates were available, positive deer from outlying areas marking the geographic limits of the core habitat occupied by elk were also included, making a total of 39 individuals. To contextualise these data, 78 randomly chosen samples (from 1994 to 2013 that fell outside of the previous criteria) were sequenced from the archived list of infected cases. All cattle herds with bTB cases in the same area (three herds, nine individuals) were selected as were cases from two herds that were identified as breakdown sources through trace out investigations. In total we identified isolates from 5 elk, 117 deer and 12 individual cattle (Figure 1). Samples from all individual species were collected in the period between 1996 and 2013. The distribution of isolates by year and species is presented in Table S1. Cattle and elk were found positive either in the same year or cattle herds were found infected 1-3 years after elk infected cases. Population size and bTB prevalence information for each host species is presented in Table S2.

2. **Whole-genome sequencing and SNP analysis**. DNA was collected from *M. bovis* cultures, libraries were prepared using NexteraXT and then sequenced on an Illumina MiSeq using 2 X 250 paired end chemistry. Multiple isolates were indexed per lane, providing approximately 50-100x coverage per isolate. Raw sequences were aligned to the reference genome AF2122/97 (Genbank accession code PRJNA89) using a Burrows-Wheeler Aligner (BWA) (Li and Durbin 2009) and Genome Analysis Toolkit 2.5.2 (GATK) (McKenna et al. 2010; DePristo et al. 2011; Van der Auwera et al. 2013). Base quality score recalibration, duplicate removal, single-nucleotide polymorphism (SNP) and indel (insertion or deletion of nucleotides in the genome) discovery and genotyping were applied across all isolates using standard filtering parameters or variant quality score recalibration according to GATK Best Practices recommendations

(McKenna et al. 2010; DePristo et al. 2011; Van der Auwera et al. 2013). Sites that fell within Proline-Glutamate (PE) and Proline-Proline-Glutamate (PPE)- polymorphic CG-repetitive sequences (PGRS) were filtered, as well as SNP positions with a phred-scaled quality (QUAL) score for the alternate non-reference allele lower than 150 and allele count (AC) equal to 1 (see https://github.com/USDA-VS/snp_analysis for bioinformatics scripts and Table S3 for sequencing statistics). Integrated Genomics Viewer (IGV) was used to visually validate SNPs, and SNPs with mapping issues or alignment problems were manually filtered.

3. **Phylogenetic reconstruction of *Mycobacterium bovis*.** Evolutionary relationships among *M. bovis* isolates were generated using a Bayesian coalescent Markov chain Monte Carlo (MCMC) analysis in BEAST 2 (Bouckaert et al. 2014). To verify the existence of temporal signal in the data, we explored the temporal structure of the sequences using the software Tempest (Rambaut et al. 2016) and performed a tip-date randomization test (Firth et al. 2010), where we looked for the absence of overlap between the 95% credible interval of the original rate estimate and any of the date-randomized datasets (Ramsden, Holmes, and Charleston 2008; Duffy and Holmes 2009; Firth et al. 2010; Duchêne et al. 2015) (see Text S1 for analysis description). We used a marginal likelihood estimation (MLE) model selection approach (path sampling (Lartillot and Philippe 2006)) to determine the best-fit nucleotide substitution, clock and demographic models. Two nucleotide substitution models (Hasegawa, Kishino and Yano (HKY, (Hasegawa, Kishino, and Yano 1985)), and General Time Reversible (GTR, (Tavare 1986)) that were both supported by the Bayesian information criteria model selection jModeltest 2 (Darriba et al. 2012)) were chosen for model selection. Four molecular clock models (strict, relaxed normal, relaxed exponential, and random local) were evaluated in combination with three coalescent demographic models (constant population size (Drummond et al. 2002; Kingman 1982), Bayesian skyline (Drummond et al. 2005), and Bayesian extended

skyline (Heled and Drummond 2008)). Model performance was evaluated by MLE based on the average of two runs of path sampling and paired comparisons (of all models to the first combination: HKY, constant population size and strict clock) of marginal likelihoods using Bayes Factor (Kass and Raftery 1995). The best-fit model combination was: HKY nucleotide substitution model with a gamma-distributed rate variation (which enables the evolutionary rate to vary amongst sites), the uncorrelated exponential relaxed clock model (which allows each branch of the phylogenetic tree to have its own evolutionary rate), and an extended Bayesian skyline model (which estimates the demographic function directly from sequence data without the requirement of pre-choosing the model dimensionality). Two independent MCMC analyses were run for 100 million generations and posterior distributions were sampled every 10,000 generations. Model parameters were assessed for convergence and satisfactory effective sample sizes (>200) in Tracer V1.6 (Rambaut et al. 2014). These runs were combined in LogCombiner v2.4.8 (Drummond and Rambaut 2007) where trees were subsampled as well, and a maximum credibility tree was estimated (after discarding the first 10% of trees as a burn-in) using TreeAnnotator v2.2.0 (Drummond and Rambaut 2007). We estimated the overall *M. bovis* evolutionary rate and the Most Recent Common Ancestor (MRCA) dates for all individual clades. In this study, we defined a phylogenetic clade as a cluster of individual isolates that was evolutionary distinct from other clusters and also highly supported (≥0.95).

4. **Spatial and genetic distances between isolates**. To illustrate the spatial distribution of each phylogenetic clade, the spatial positions of each isolate were plotted and a convex hull (i.e. the smallest polygon incorporating a given set of points) was drawn around each estimated clade. To check how clades are distinctively clustered in space, the (Euclidean) spatial distances between isolates in the estimated and randomly generated clades were computed. For every pair of clades being compared, 1,000 random points were chosen from each, and spatial distances

were computed per random pairs of isolates. This analysis was repeated for the random (permuted) clade assignments and plotted for all clade pairwise comparisons. A k-means analysis was also performed to identify four spatial clusters of isolates. If the clades are distinctively clustered in space, then there will be a large overlap between the spatial positions of these clusters and of the estimated clades. The minimum spatial and genetic (number of different sites between sequences) distances were computed between each pair of isolates and separated by host species interaction. The spatial analyses were implemented in R (RCoreTeam 2014) and used the packages maps (Becker and Wilks 2016), maptools (Bivand and Lewin-Koh 2017), and rgeos (Bivand and Rundel 2017), while the genetic analysis used the R package ape (Paradis, Claude, and Strimmer 2004).

5. **Ancestral state host reconstruction using discrete traits**. Host species were modelled as a discrete trait over the *M. bovis* genealogy by ancestral state inference using Discrete Ancestral Trait Mapping (DATM) in BEAST 2 (Bouckaert et al. 2014). This approach allowed us to estimate the probability of internal node states and branches being associated with a specific host (and as such the most likely source of infection within the sampled *M. bovis* population in elk, deer or cattle), based on relationships among the host states at the branch tips (from the sampled isolates). Host state posterior probabilities (PP) were reported for ancestral nodes up to the most recent common ancestor. All nodes were annotated with their PP values. The three-state analysis (elk=5, deer=117, and cattle=12) estimated over time the posterior probability that a pathogen transition rate between a particular pair of discrete host states was positive. If this probability is high, then the data strongly support a model (evaluated by Bayes' factors) in which a direct pathogen transition between that particular pair of host species can occur. Similarly, the relative transition rate between that pair of host species was also computed. Two MCMC analyses were run for 100 million generations, sampling every 10,000 generations. The

BEAST output was analysed using the Tracer v1.6 program (Rambaut et al. 2014). The phylogenetic trees produced by BEAST were subsampled in LogCombiner and annotated using TreeAnnotator v2.2.0 (Drummond and Rambaut 2007), and the maximum clade credibility tree was visualized using the FigTree v1.3.1 program (Drummond and Rambaut 2007). The estimated posterior probabilities of support of transitions between pairs of host species were plotted for all cases. For the cases where the probability was high, providing strong evidence of direct transition between a particular pair of host species, the mean posterior probability of rate changes was presented.

6. **Phylogenetic tip-host species, down-sampling and extra-elk permutation tests.** To validate the results associated with host state interactions where our models support pathogen transitions between particular pairs of host states, we performed three phylogenetic tests: a) a phylogenetic tip-host species permutation to investigate the extent of pathogen genetic signal in the host populations, b) a down-sampling test to study the impact of different numbers of isolates in host species interactions**,** and c) a phylogenetic tip-elk permutation test to check the impact of extra elk in host species interactions. In a) this test involved generating 10 new randomized data sets by permutation of sampled host species, performing DATM analysis for each new file with the same settings as section 5, and comparing parameter estimates (probability of pathogen transition between host species) obtained with the initial data set versus the randomized ones. In b) to test the influence of the uneven number of isolates per host species on the results of our analysis, we generated four types of data sets with a different number of sampled host species each (chosen randomly): Subsample A corresponds to 10 data sets of 5 elk (all elk isolates), with 5 random samples from each of the available cattle and deer isolates; subsample B corresponds to 10 data sets of 5 elk, 12 cattle (all cattle isolates) and 12 deer randomly sampled from the 117 deer isolates available; subsample C corresponds to 10 data

sets of 5 elk, 12 cattle, and 36 deer randomly sampled from the 117 deer isolates available; and subsample D corresponds to 10 data sets of 5 elk, 12 cattle, and 76 deer randomly sampled from the 117 deer isolates available. New DATM analyses with the same setting as section 5 were performed for each one of the 10 files of each data set type. Parameter estimates from the 10 analyses in each dataset were combined and compared with the original data. These results were shown in boxplots. In c) to identify the effect of under-representation of infected elk in the dynamics of the disease, we have extended our analyses with simulations of 1 and 2 extra elk in the population. We focused on the clades where we have elk and cattle isolates (clades 1-3) and added n elk to our dataset (by randomly replacing the host species labels of n deer by n elk). We repeated this analysis 10 times for n=1 and n=2 (testing the effect of having 6 and 7 elk) and computed the probability of support for pathogen transition between each hot species. We compared the results to the original one (with 5 elk).

Results

1. **Phylogenetic reconstruction of *Mycobacterium bovis***. Whole-genome sequencing of the 134 *M. bovis* isolates sampled between 1996 and 2013 from multiple hosts (deer, elk and cattle) identified 391 SNPs. An analysis using Tempest supported by tip-date randomization test support the existence of a strong temporal signal in the data (see Text S1 and Figure S2). The time-measured phylogeny, estimated under an uncorrelated relaxed exponential clock and an extended skyline demographic model using BEAST 2 (Figure S3, Table S4), shows three major clades (Figure 2). None of the clades could be distinguished from the others solely by the sampling time of its isolates, nor the area from where they were sampled (Figures 2-3). The spatial distribution of the different clades overlapped with each other to the point where there was no difference between spatial distances calculated between isolates from different clades when these were correctly or randomly assigned (Figure 3A-B). Furthermore, there was no

visible relationship (Figure 3-C) between the spatial pattern generated by the three clusters (identified by within group sum of squares in k-means, Figure 3-D) and the one generated by the three clades. These results suggest that different lineages have been co-circulating in the sampled area. The mean evolutionary rate of *M. bovis* was estimated to be 0.37 substitutions per genome per year (95% HPD: 0.24-0.51 substitutions per genome per year) (Figure S4), which is consistent with previous *M. bovis* studies in other settings and with other wildlife hosts (Biek et al. 2012; Trewby et al. 2016; Crispell et al. 2017).

2. **Investigation of interspecies transmission**. The ancestral host state reconstruction showed that multiple host species were distributed within the different clades, indicative of interspecies transmission (except in clade 3 where deer are the only species present, Figure 4). The clustering patterns of host species observed in clade 2 indicate a strong probability of intraspecies transmission of bTB in the sampled cattle population, while the individual clusters of two elk and two cattle isolates suggest either there are intraspecies transmission events of bTB in the sampled elk and cattle populations, or the infection in each species is due to other common sources. The wide distribution of deer over all the clades suggests that intraspecies transmission is occurring in the sampled deer population and that deer also play an important role in the transmission to other species. State transitions between deer and cattle, and deer and elk were shown to have strong support (PP=0.996 and 0.989, Table 1), but the transition between cattle and elk was poorly supported (PP=0.391, Table 1). When compared to all isolates, cattle and elk isolates were never the closest genetically or spatially to each other (Figures S5-6).

3. **Phylogenetic tip-host species, down-sampling and extra-elk permutation tests.** To check the veracity of our results we performed host-tip randomization, down-sampling and additional

376 elk analyses. Figure 5 shows that the estimated interactions between deer and elk, and cattle
377 and elk with the real data (presented by stars) differ from the ones estimated with the
378 randomized data sets (presented by boxplots), with the exception of the estimated interactions
379 between cattle and deer from the real and random data sets, which overlap with each other. This
380 overlap suggests that pathogen migration between these two species is consistent with it being
381 a random process. If this is the case, then increases of bTB in cattle may simply be attributable
382 to increases in deer population densities and infection levels. The sensitivity analyses to show
383 the effect of sample size (of each host species) on the interspecies interaction, show that this
384 measure only influences our results under extreme down-sampling (dataset A). However, with
385 lesser but still substantial down-sampling of data (B, C and D; which have variations in sample
386 size for each host species), our analyses show a similar pattern to the original data: strong
387 support for interactions between deer and cattle and deer and elk, and low support for
388 interactions between cattle and elk (Figure 6, Table 1). Finally, the addition of 1 or 2 "elk"
389 samples to our pool of isolates (by replacing deer isolates) were shown to be insufficient to
390 change our results (Figure S7).
391
392 **Discussion**
393 This analysis is one of the few genomic studies examining bacterial transmission at the wildlife-
394 livestock interface (Kamath et al. 2016) in the United States and highlights the important role
395 that genomics and phylodynamic approaches play in improving our understanding of fine scale
396 transmission patterns. Using *M. bovis* genomic data from different host species with a time
397 frame of 17 years, we showed that, even with a slow, highly variable substitution rate, WGS
398 has remarkable power to identify the likely roles of different host species in the transmission
399 dynamics of endemically circulating diseases, independent of other epidemiological evidence.
400 However, with chronic diseases such as bTB (months to years to show signs of infection), we

have to consider the possibility of infections that were missed during testing, and that we could be underestimating the amount of transmission. Furthermore, any interpretation of the results should take into consideration the assumptions and limitations of the data and models used in the study. DATMs assume sampling numbers are informative of the underlying prevalence of the disease in different hosts. We have a low sampling number of isolates from elk and cattle and a large number from deer, however, we present information on population sizes and number of sampled and infected cases for each species, demonstrating that our samples are related to the underlying levels of the disease for each host species. Furthermore, the sampling effort in the elk is very high given the proportion of individuals tested relative to the total population size. These models also assume homogeneous mixing in the underlying sampled population, which was addressed by choosing high number of random deer isolates. However, for future studies with structured populations, the adoption of methods like the Bayesian Structured Coalescent Approximation (BASTA) (De Maio et al. 2015), which relaxes that assumption, might be more suitable. Michigan has an unprecedent surveillance system for elk – since their introduction to the state in 1918, they have been heavily managed to ensure a healthy and stable population size (~800 individuals) (MDNR3, n.d.) but even with such a system a few infected cases might have been missed. We showed that even if infected elk were under sampled by 40% compared to deer (i.e. two more infected elk), the additional interactions do not alter the key conclusion; however additional analysis would be needed to determine how many more elk would be needed to see an effect. Spatial analyses show that even with the addition of a large random sample of infected deer, disease transmission events occur at small spatial scales with circulation of distinct strains. The spatial overlap of the clades supports the idea that the pathogen population is well mixed (at this scale). Furthermore, *M. bovis'* low and variable substitution rates can sometimes challenge accurate estimations of evolutionary rates. Our estimates of *M. bovis* evolutionary rate for this sampled population is similar with the ones

found in other studies with different organisms (Biek et al. 2012; Trewby et al. 2016; Crispell et al. 2017).

Our results suggest that in the Michigan bTB endemic situation to date, elk so far are unlikely to be a maintenance reservoir. The lack of support of pathogen transition between elk and cattle also suggest that elk do not have an active role in the transmission of *M. bovis* infections to the neighbouring livestock populations. These genomic findings support conclusions based on previously reported pathologic and epidemiologic data (O'Brien et al. 2006, 2008). Overall, the topology of the *M. bovis* phylogeny indicated the existence of interspecies transmission events, with the presence of multiple host species interspersed within clades. Deer isolates were found in all 3 clades, showing that in our selection of isolates there is higher genetic diversity circulating in this host population than in any other, adding to the accumulated evidence from previous ecological studies that deer are a significant source of bTB in livestock and other wildlife species. However, the clustering of isolates by host species suggest the majority of transmission events were occurring either within species, or from a common source, (exposure to the sampled deer population or other intermediate hosts (Lavelle et al. 2016)), or both. For the Michigan elk population, if any of the clustering is due to intraspecies transmission, this is a new and epidemiologically significant finding. If the clustering of infected elk noted in Clade 2 of our study is due to elk-to-elk transmission, it may be that transmission has not yet reached a sufficient threshold for self-maintenance. That said, if intraspecies transmission has occurred at all it should serve as warning to state wildlife managers of the necessity of preventing further introductions of *M. bovis* into that valuable population. Thus, human-caused aggregations (such as recreational feed and bait sites intended for deer) that act as sources of indirect contact between elk and deer must not be allowed to occur.

In Canada, wild elk have proven to be competent maintenance hosts for bTB (Manitoba Agriculture Department, n.d.; Shury and Bergeson 2011; Shury 2015). Reasons why elk in Canada are maintenance hosts and in Michigan they seem not to be, are not clear, however, are likely to be related to different population sizes, densities, social behaviour and home ranges. In Canada, populations are likely to be larger and denser and composed of multiple groups, while in Michigan they are smaller, and without structured groups and they only overlap slightly with the deer endemic area (Walsh 2007; Shury and Bergeson 2011; Shury 2015). Other factors such as management practices, historical facts of bTB (especially, how long the area has been infected), habitat quality, and opportunities to have inter- and intra- species contact may also play a role in the persistence of *M. bovis* in these populations.

We also demonstrated via DATM, and genetic and spatial isolate pairwise comparisons, that there is very low support for transition events between elk and cattle. This might be due to the fact that the elk population is an order of magnitude smaller than the deer population, which may decrease the probability of contact with livestock. In addition, much of the core elk range in Michigan is composed of publicly-owned lands that are relatively remote from livestock operations. These findings suggest that bTB eradication efforts in the elk population are currently unnecessary due to the low probability of spillover to cattle, and that the major focus should continue to be in controlling the disease in the endemic deer population. However, should the elk population increase, this could enhance their role in the maintenance of bTB in Michigan. Furthermore, the possibility of other species acting as intermediate hosts and being involved in the transmission of *M. bovis* to the cattle population remains possible. Other spillover hosts including black bear (*Ursus americanus*), bobcat (*Felis rufus*) coyote (*Canis latrans*), red fox (*Vulpes vulpes*), raccoons (*Procyon lotor*), and opossums (*Didelphis*

virginiana) have been shown to be bTB spillovers in this area (Bruning-Fann et al. 2001; Walter et al. 2013; A. R. Berentsen et al. 2011). It could be though direct contact (however unlikely (Scott D. Fitzgerald et al. 2003)), or through environment contamination. Both raccoons and opossums are found to share communal dens resulting in increased interaction when resources are abundant such as around feed stockpiled for livestock (Palmer, Waters, and Whipple 2002; Atwood et al. 2009), and when they have a chance, they use the same stored feed, water sources, and feed being consumed by cattle (Bruning-Fann et al. 2001; Atwood et al. 2009; Walter et al. 2013), increasing the chances of contamination. More studies on these populations would help to understand their contribution to the spread of bTB.

In Michigan, bTB has been a concern of management by both wildlife and agriculture agencies for two decades. Prospects for eradication are uncertain, and the ongoing costs of disease management necessitate the use of innovative methods to inform management decisions. By providing insights into reservoir status and the likelihood of interspecies transmission, genomic analyses such as this supplement traditional epidemiologic and pathologic data, advancing efficient and effective use of both bTB surveillance and control resources.

Data Accessibility

The raw sequence files (FASTQ) were submitted to the NCBI Sequence Read Archive under the Bioproject accession number: PRJNA251692. The individual isolates can be accessed under the following Biosample accession numbers: SAMN07339977 - SAMN07340029 and SAMN10254813 – SAMN10254893. Information about metadata associated to each isolate is in Table S3. The R scripts used for this publication are freely available on the following Github link: https://github.com/lsalvador/WGS_Michigan_Project.

Author Contributions

LCMS designed the study, performed research, analysed data, and wrote first draft. DJOB designed the study, collected data from wildlife, contributed to interpretation and wrote first draft. MKC designed the study, collected data from wildlife, contributed to interpretation and performed GIS analysis. RRK developed the initial project proposal, designed the study, advised on its implementation and contributed to interpretation. AS and SC revived archived *M. bovis* isolates. SRA sequenced and provided genomic data and livestock metadata. TPS performed bioinformatics analysis. YTG developed the initial project proposal and provided input on the study design. JC provided useful insights on molecular evolution analysis and performed the spatial analysis. MKC, SRA, TPS, JC, YTG and RRK provided comments on the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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Tables and Figures

Host species interaction	Estimated posterior probability of transition between host species (symmetric)	Estimated absolute transition between host species (event/genome/year)	Strength of support by Bayes' factor (BF > 3: well supported BF > 10: very strong support)
Cattle-Deer	**0.996**	**0.012**	**28.37**
Cattle-Elk	0.391	0.011	0.073
Deer-Elk	**0.989**	**0.011**	**10.24**

Table 1. Evidence of pathogen transition between host species. Results from a discrete ancestral trait mapping analysis.

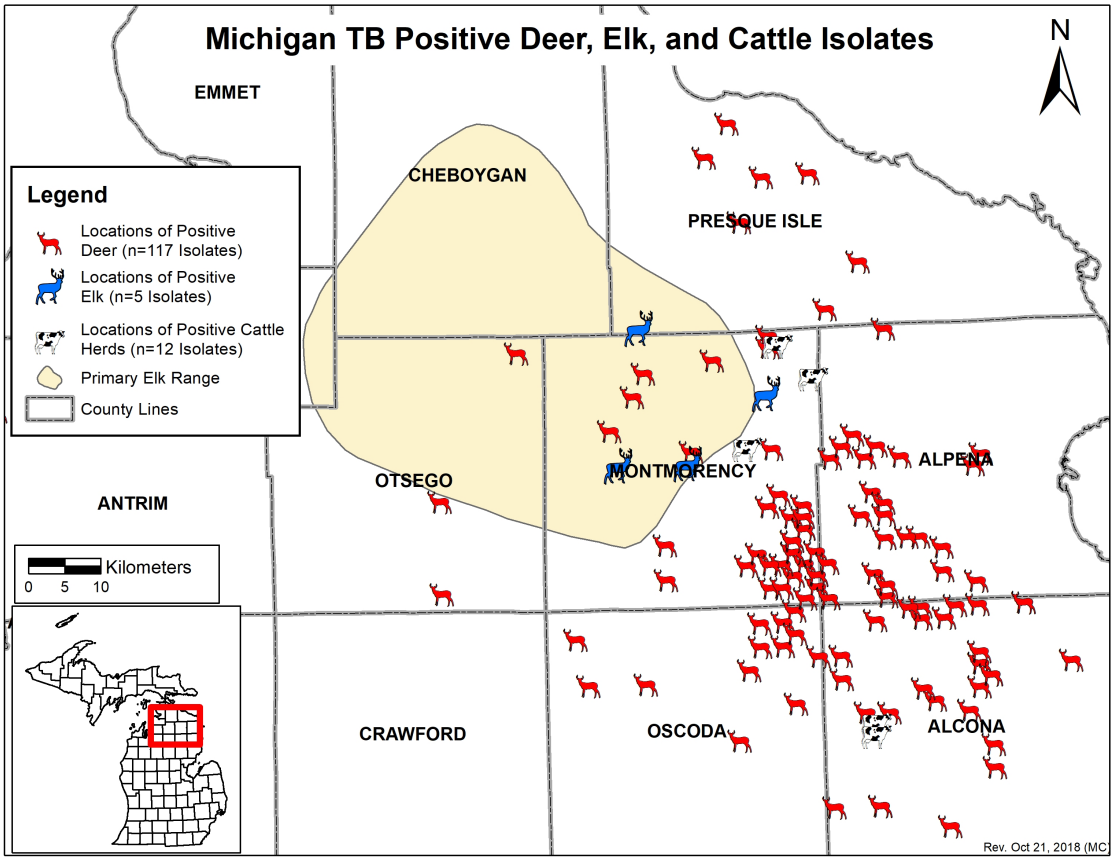

Figure 1. Study area in northeastern Lower Peninsula of Michigan, USA with locations of bovine tuberculosis positive animals. Positive samples from deer that were spatially and temporally close to each positive elk and from the margins of the occupied elk range were selected for inclusion from among available archived isolates (39). This dataset was extended with 78 more positive deer samples randomly chosen from the available archived isolates. Positive cattle herds in the same area (9) were also selected together with trace backs of infected individuals from other herds (3). In total isolates from 5 elk (from 2000 to 2006), 117 deer (from 1996 to 2013) and 12 individual cattle (from 2000 to 2009) from 3 neighbouring herds

and 2 other herds identified by trace backs. The isolates were sampled from 8 counties: Montmorency, Presque Isle, Otsego, Oscoda, Alpena, Alcona, Emmet and Antrim. Isolates that were collected from the same host species in the same location are overlapped in the figure.

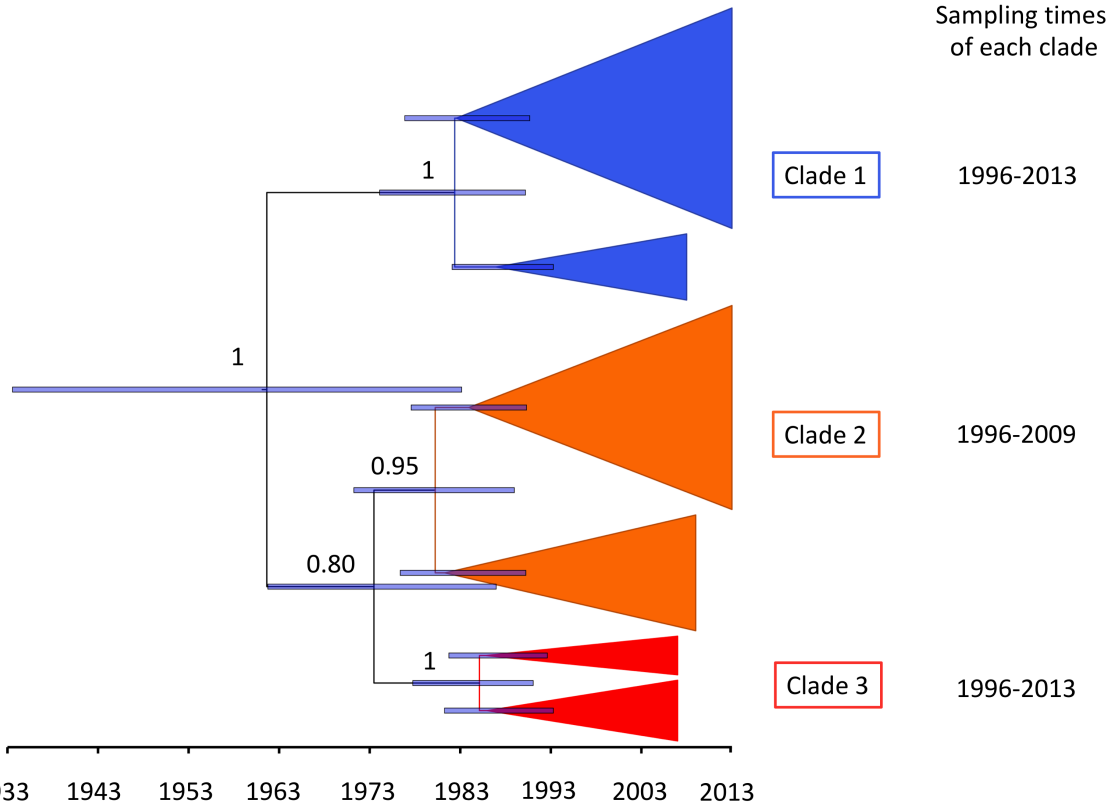

Figure 2. Time-calibrated maximum clade credibility tree of *Mycobacterium bovis* isolates. Four *M. bovis* clades (C1-C3) were identified through Bayesian phylogenetic analyses of 117 *M. bovis* isolates sampled between 1996 and 2013 under an uncorrelated relaxed exponential clock and extended skyline demographic model. Posterior support for major nodes is shown with grey bars indicating the 95% highest posterior density intervals for node date estimates.

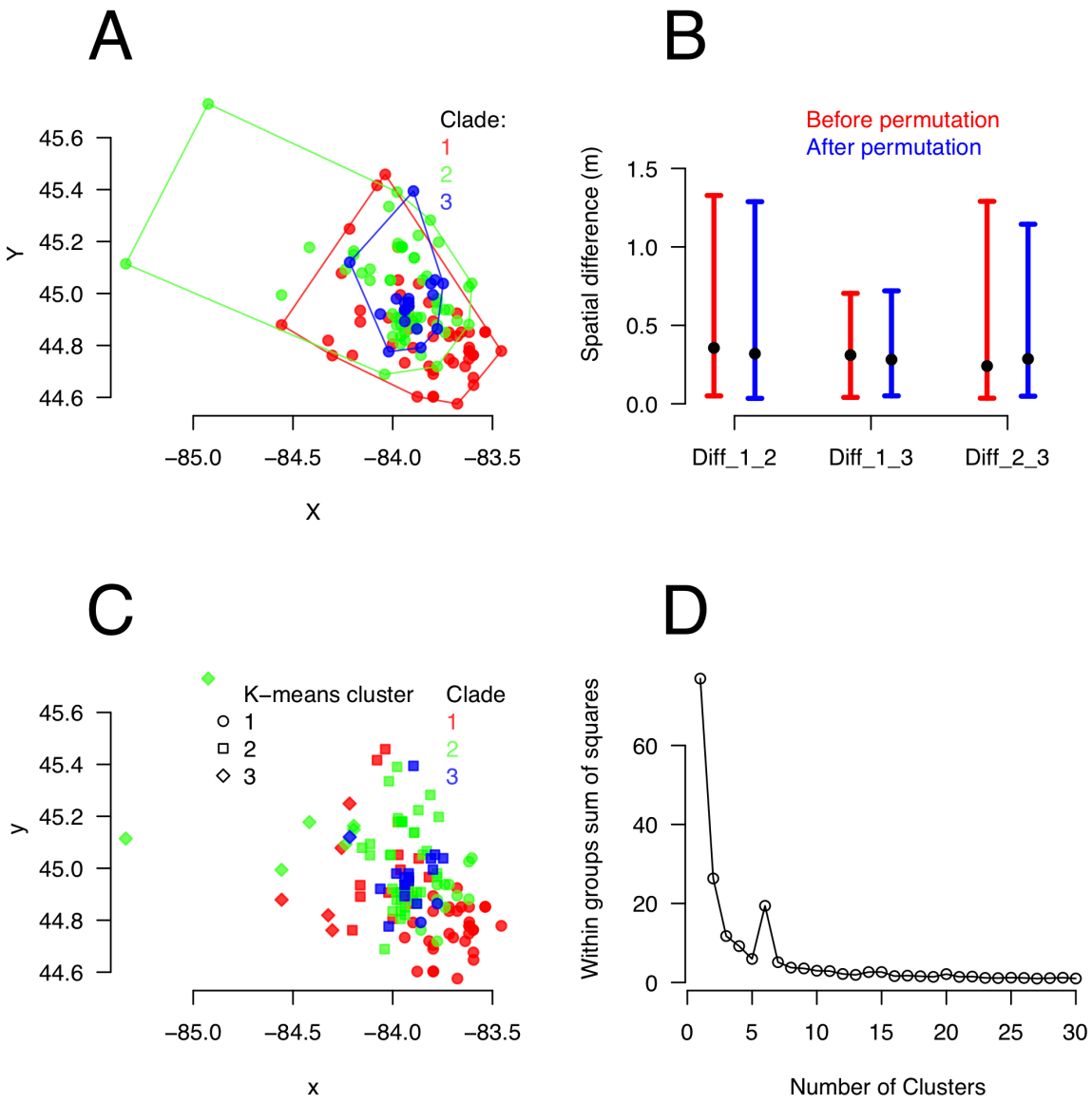

Figure 3. Spatial analysis of *Mycobacterium bovis* isolates. A. Spatial analysis of distribution of *M. bovis* clades identified by Bayesian phylogenetic analysis. Each polygon represents the minimum convex polygon of the sampled locations of the isolates of each clade. B. Comparison of spatial distances between estimated and permuted clades. For every pair of clades being compared we have randomly selected 1000 isolates from each. For each random pair of isolates we calculated the spatial distance between them. This analysis was repeated with random (permuted) clade assignments. C. K-means analysis with 3 clusters (represented by symbols) versus 3 clades (represented by colors). D. Optimal number of clusters estimated by within group sum of squares (distances between individuals within each cluster). The optimal number of clusters will be the number after which within cluster differences become minimal; here this occurs after ~ 3 clusters.

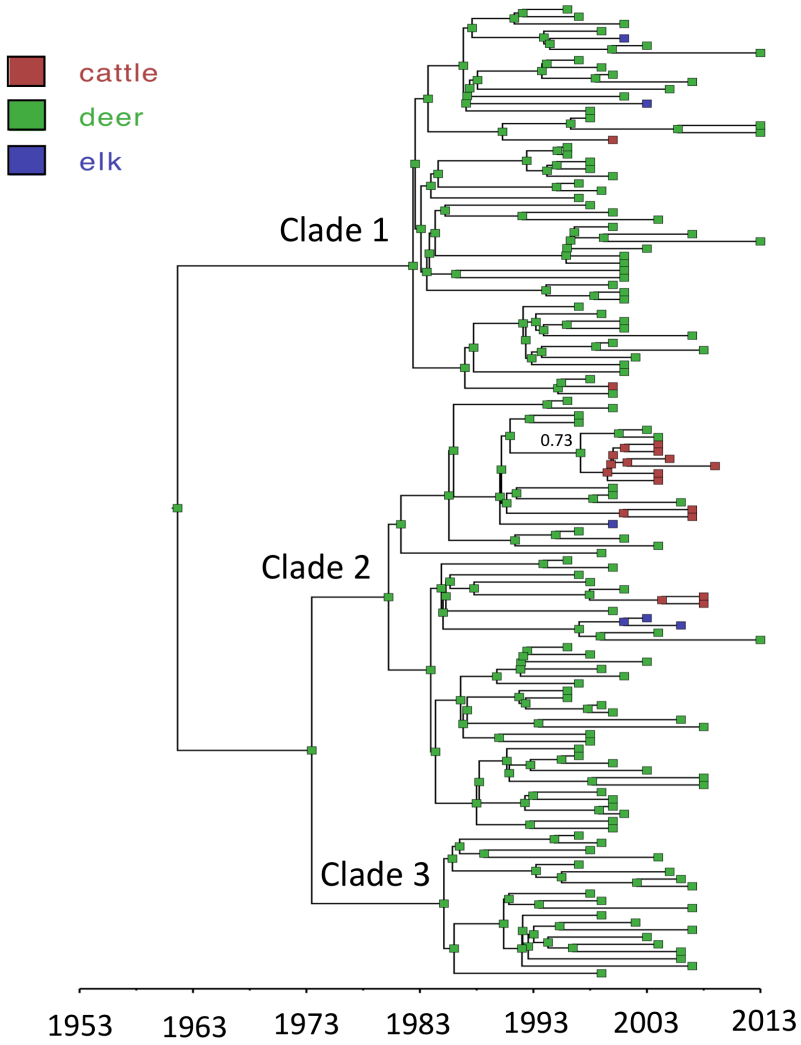

Figure 4. Ancestral host state reconstruction over the *Mycobacterium bovis* phylogeny. Maximum credibility tree was estimated under a model of symmetric host species transitions. Host state posterior probabilities (PP) are reported for ancestral nodes up to the most recent common ancestor. All nodes have PP values above 0.95 and only one (with PP=0.73) is annotated. Host species are represented by squares with the following colour labels (cattle=red, deer=green, elk=blue).

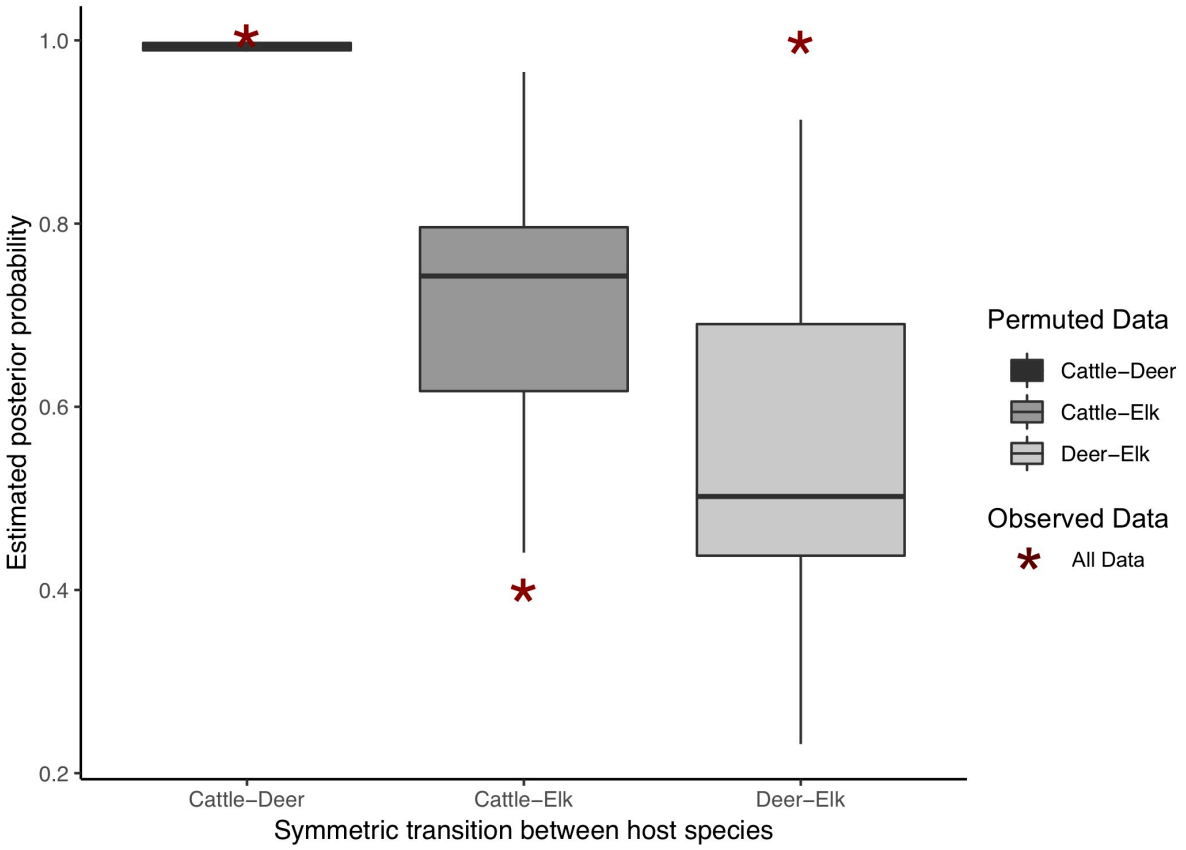

Figure 5. Comparison of the estimated posterior support of direct host species transition between permuted and observed data. The estimated posterior mean probability of each host species interaction is the posterior probability that a particular transition rate is positive. If this probability is high, the data strongly support a model in which there is direct pathogen transition between that particular pair of host species. The posterior means were estimated via a Discrete Ancestral Trait Mapping performed in BEAST v2. The 'Permuted data' correspond to the posterior means of 10 BEAST runs of each interaction after permuting the host species labels each time. The 'Observed data' correspond to the posterior mean of each interaction using the observed data.

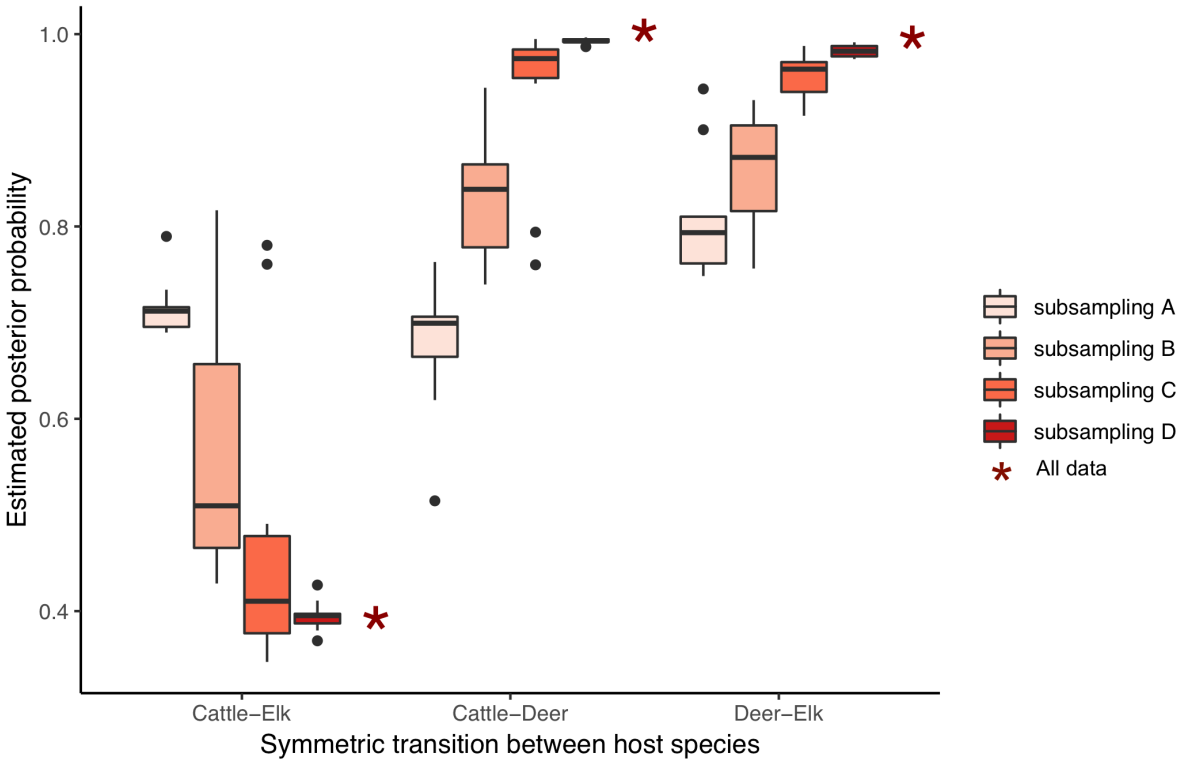

Figure 6. Comparison of the estimated posterior support of direct host species transition between subsampled and observed data. The estimated posterior mean probability of each interaction is the posterior probability that a particular transition rate is positive. If this probability is high, then the data strongly support a model in which there is direct pathogen transition between that particular pair of host species. The posterior means were estimated via a Discrete Ancestral Trait Mapping performed in BEAST v2. The 'Subsampled data' correspond to three subsets of 10 files where the different isolates found in each species were randomly chosen to be part of the new data set. Subsample A corresponds to isolates sampled from five elk ('Elk'), five randomly chosen cattle ('Cattle'), and five randomly chosen deer ('Deer'); Subsample B corresponds to isolates sampled from five elk, nine cattle, and nine randomly chosen deer; and Subsample C corresponds to isolates sampled from five elk, nine cattle, and twenty four randomly chosen deer. The 'All data' correspond to the posterior mean of each host species interaction output by one DATM analysis using all of the observed data, which consists of five elk, twelve cattle, and 117 deer.

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